Nucleotide and the types of nucleotide are terms well known in the art. Those skilled in the art readily understand what a nucleotide is and that there is a wide range of them, including naturally occurring and non-naturally occurring nucleotides. There is not need for Applicants to provide definition in the specification for such well known terms. Thus, Applicants submit that the meaning of the term "type of nucleotides" is clear and that there is no indefiniteness in the recital of this term in the rejected claims. Withdrawal of the instant rejection is respectfully requested.

Claim Rejection: 35 U.S.C. § 102(b)

Claims 1, 2, 4-13, and 16 are rejected as allegedly anticipated by Dower et al. (U.S. Patent No. 5,547,839). The Examiner says that Dower et al. teach a method for sequencing nucleic acid molecules, which comprises the steps of: (i) providing at multiple locations a plurality of nucleic acid molecules which have the same sequence as one another; (ii) providing at each location with a nucleic acid polymerase and a given labeled nucleotide under conditions that allow primer extension; (ii) detecting whether or not the labeled nucleotide has been used for primer extension at each location by determining whether the label present on said nucleotide has been incorporated into the extended primer; (iv) detecting the nucleotides used per extended primer if said nucleotide have been used in primer extension; and (v) obtaining the sequence of the nucleic acid molecule by referencing to the signal depicted at each location and the identifying nucleotides used in the primer extension at each location (Office Action, paragraph 7, at pages 3-4).

In maintaining the instant rejection, the Examiner also says that "Dower et al. also teach wherein the primer extension step comprising multiple labels is repeated" (Office Action, paragraph 7, at page 4; emphasis added). This rejection is respectfully traversed for the reasons stated below.

The Examiner is advised that the sequencing scheme discussed in Dower et al. is different from the presently claimed methods. The basic distinctions are set forth in the following paragraphs. However, if the Examiner insists that the presently claimed methods are anticipated by Dower et al., Applicants respectfully invite the Examiner to telephone Applicants' undersigned representative for further clarification.

First, it is important to note that Dower repeatedly teaches that the removal of a label from a nucleotide used in primer extension is needed before adding a further labelled nucleotide. This contrasts with the present invention, whereby a plurality of labels are incorporated in a growing strand by primer translation (or nick translation) and the need to remove labels is avoided. Such a feature of the present invention is reflected in Step (c) of independent claims 1 and 17 (as amended) which recite that the extended primers each comprising a plurality of labels. Applicants have amended the claims to make it clearer that each of the extended primer comprises a plurality of labels.

The above noted distinction is also disclosed in the subject specification. For example, in the last paragraph of page 6, it is stated:

> "The present invention allows labelled nucleotides to be incorporated in a stepwise manner in a nucleic acid molecule via primer extension. However the presence of one labelled nucleotide in the molecule does not prevent other labelled nucleotides being detected (even if adjacent labelled nucleotides are the same). Thus, unlike many prior art parallel sequencing methods, such as those disclosed in W093/21340 and DE-A-4141178, there is no need to remove a label from a polynucleotide chain before a further labelled nucleotide is added (although in some embodiments, it may be desired to remove labels periodically). A plurality of labels can therefore be incorporated into a nucleic acid molecule via primer extension and can be detected in situ." (emphasis added)

By contrast, Dower et al. do not teach a sequencing scheme in which each of the extended primers comprises a plurality of labels. Rather, the Dower et al. scheme requires removal of the label after each extension cycle. As illustrated by the various examples below, removability of the label is said to be an important feature of the Dower et al. scheme.

For example, at Col. 15, lines 52 and 53 of Dower, it is stated:

> "One important functional property of the monomers is that the label be removable." (emphasis added).

Indeed the importance of removal of the label is stressed throughout Dower. Reference can also be made, for example, to the various figures. In all of the figures showing

labelling after initial incorporation of a label, the label is removed before a further label is added:

(1). In Figure 1 of Dower, a degradative procedure is illustrated, whereby a terminal residue of an immobilized polymer is labelled and the labelled terminal residue is then removed before subsequent steps of end labelling and removal of terminal residues can be performed (compare Fig 1B with Fig 1C and Fig 1D);

(2). In Figure 2, a synthetic procedure is illustrated, whereby a labelled residue is used to extend an immobilized polymer at a terminus thereof. A labelled residue is used in each extension step, but the label is removed before any further extension steps (compare Fig 2C with Fig 2D);

(3). In Figure 8A, a synthetic procedure is also illustrated, this time showing the use of a labelled nucleotide (G*) in primer extension. The label is incorporated in step [1] and is removed in step [2] (Note the absence of the asterisk next to G following step [2]). Figure 8B provides a general flowchart for primer extension using labelled nucleotides. It can be seen that a single nucleotide is used in elongation, followed by a scanning step and a step to remove label before further cycles are performed;

(4). Further, Figures 10A and 10B are analogous to Figures 8A and 8B, but are in respect of a degradative scheme. It can be seen that in step [1] of Fig 10A a terminal C residue of an immobilized polymer is labelled and that the labelled terminal residue is then removed before further label is added in step [4].

In addition to the figures of Dower, reference can also be made to the corresponding parts of the description, where removal of label is repeatedly stressed.

In light of the above remarks, it readily apparent that Dower et al. do not teach the presently claimed methods. Withdrawal of the instant rejection is respectfully requested.

Claim Rejection: under 35 U.S.C. § 103

Claims 15, 17, 21, and 23 are rejected as allegedly unpatentable over Dower et al. in view of Levin B (Genes IV, Oxford University Press, New York, December 1990). The Examiner cites to Dower et al. for the alleged teachings as discussed above. The Examiner cites to Levin as teaching a general method for providing nicks into double stranded nucleic

acid molecules by nick translation. As such, the Examiner says claims 15, 17, 21, and 23 are obvious of the cited reference. Applicants respectfully traverse this rejection. While the instant rejection is made to claims 15, 17, 21, and 23, Applicants submit that the following discussions establish that, in addition to claims 15, 17, 21, and 23, the other claims pending in the application (i.e., claims 1, 2, 4-14, and 16) are also non-obvious over the cited art.

A. The subject invention is different from and nonobvious over Dower et al.

As noted above, the subject invention does not require removal of label after each extension step. As a result, claims 1 and 17 recite that the extended primers each comprising a plurality of labels. Claim 21 has also been amended to more clearly point out such a feature of the subject invention. On the other hand, Dower et al. discuss a sequencing scheme that requires removal of label after each extension cycle. As the removal step is an important feature of the Dower et al. method, it would clearly not have been obvious to modify the teaching of Dower et al. so as not to remove the label as does the presently claimed invention.

Consistent with the removal of label after each extension cycle in Dower et al, a further difference between the present invention and Dower et al. is that Dower requires the presence of a blocking agent to ensure that no more than one label becomes incorporated at a given stage of primer extension (or nick translation). Reference can be made, for example, to the following parts of Dower et al:

> "Note that only one A monomer is added." (Col. 7, line 23)
>
> "Again note that only one, and not successive B' monomers, is added." (Col. 7, lines 29 to 30)
>
> "However, it is very important that the agents used do not remove or add successive monomers. This is achieved in the degradative method by use of highly specific reagents. In the synthetic mode, this is often achieved with removable blocking groups which prevent further elongation." (Col. 7, lines 52-57)
>
> "The primer is elongated one nucleotide at a time by use of a particular modified nucleotide analog to which a blocking agent is added and which prevents further elongation." (Col. 14, lines 50-53)

"Reaction 2 is performed, which removes both the label and blocking group." (Col. 15, lines 13 and 14)

"Reaction 1 elongates the primer by a single labeled blocked nucleotide." (Col. 15, lines 21 and 22)

"Usually, a nucleotide will be blocked at the 3' hydroxyl group where successive nucleotides would be attached. In contrast to a dideoxy nucleotide, typically the blocking agent will be a reversible blocking agent thereby allowing for deblocking and subsequent elongation." (Col. 15, lines 35 to 40)

"Nucleotide analogs used as chain-terminating reagents will typically have both a labeling moiety and a blocking agent while remaining compatible with the elongation enzymology. As the blocking agent will usually be on the 3' hydroxyl position of the sugar on a nucleotide, it would be most convenient to incorporate the label and the blocking agent at the same site, providing for a single reaction for simultaneous removal of the label and blocking agent. However, it is also possible to put a label on another portion of the nucleotide analog than the 3' hydroxyl position of the sugar, thereby requiring a two-step reaction cycle for removing the blocking and labeling groups." (Col. 15, line 62 to Col. 16, line 6)

"As all of the growing chains have blocked nucleotides, no elongation takes place beyond a single nucleotide. The N2 nucleotides provide a specific label, detected in the scanning step. After determination of the incorporated label, the label may be removed or destroyed, and those irreversibly terminated growing chains become permanently removed from further participation in the sequencing process." (Col. 16, lines 45 to 51)

"Next, the reversible blocking moiety is removed, providing a new set of slightly longer polymers ready for the next step." (Col. 16, lines 58 to 60)

"In order to ensure that only a single nucleotide is added at a time, a blocking agent is usually incorporated onto the 3"hydroxyl group of the nucleotide." (Col. 18, lines 1 to 3)

"The blocking group should have the functional properties of blocking further elongation of the polymer." (Col. 18, lines 11 and 12)

Such extensive discussions of Dower et al. illustrate that, by employment of a blocking group, Dower et al. intend to ensure that no more than one label is incorporated in each cycle of primer extension. Such teachings of Dower et al. provide additional rationale that one would not have been motivated by Dower et al. to develop a sequencing scheme in which no removal of label is needed and the extended primer comprises a plurality of labels, as taught by the presently claimed invention. To the contrary, all the teachings and suggestions in Dower et al. would have taught away from the subject invention.

B. The subject invention has particular advantages over the cited art

A further evidence that the presently claimed methods would have been non-obvious over the cited art is that they demonstrate various advantages over the art sequencing scheme. With respect to Dower et al., the sequencing scheme of the present invention is faster and more cost-effective because it does not require blocking of nucleotides or removal of label after each extension step. Instead, the presence of one labeled nucleotide in the extended molecule does not prevent other labeled nucleotides being detected. At the same time, high sensitivity is maintained because labels are incorporated into a plurality of identical molecules present at a single location, which allow a sharp signal to be produced at each location (see, e.g., pages 6-7). For example, in some embodiments, contiguous incorporation of labeled bases can be detected wherein each nucleotide incorporation provides a cumulative increase of the same signal (see, e.g., page 18). Prior to the subject invention, an ordinarily skilled artisan, faced with the teachings of the prior art methods (e.g., the Dower et al. method), would by no means have foreseen these advantages displayed by the presently claimed methods.

Based on the above remarks, Applicants submit that claim 1 and its dependent claims, as well as claims 15, 17, 21, and 23, would have been non-obvious over the cited references. Accordingly, withdrawal of the rejections is respectfully requested.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

Respectfully submitted,

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APPENDIX. Marked-up Version of All Pending Claims
(claims not amended herewith are shown in small font)

1. **(Twice Amended)** A method for sequencing nucleic acid molecules, comprising the steps of:

a) providing at a first location a plurality of single stranded nucleic acid molecules that have the same sequence as one another and that are hybridized to primers in a manner to allow primer extension in the presence of nucleotides and a nucleic acid polymerase;

b) providing at a second location, which is different from the first location, a plurality of single stranded nucleic acid molecules that have the same sequence as one another, but that have different sequence from the sequence of the single stranded nucleic acid molecules at the first location, and that are also hybridized to primers in a manner to allow primer extension in the presence of nucleotides and a nucleic acid polymerase;

c) providing each location with a nucleic acid polymerase and a given labelled nucleotide under conditions that allow extension of the primers if a complementary base or if a plurality of such bases is present at the appropriate position in the single stranded nucleic acid molecules;

d) detecting whether or not said labelled nucleotide has been used for primer extension at each location by determining whether or not the label present on said nucleotide has been incorporated into extended primers and if said labelled nucleotide has been used in primer extension this step involves [includes a further step of] detecting how many of said nucleotides have been used per extended primer;

e) repeating steps c) and d) one or more times so that extended primers each comprising a plurality of labels are provided;

whereby the sequence of the nucleic acid molecules at the first and second

locations is obtained by reference to the number and type of nucleotides used in primer extension at these [each] location.

14. **(Thrice Amended)** A method according to claim 1, wherein the detection step is carried out without [moving] removing the nucleic acid molecules from the different locations.

17. **(Twice Amended)** A method for sequencing nucleic acid molecules, comprising the steps of:

a) providing at a first location a plurality of single stranded nucleic acid molecules that have the same sequences as one another and that are hybridized to primers in a manner to allow primer extension in the presence of nucleotides and a nucleic acid polymerase;

b) providing at a second location, which is different from the first location, a plurality of single stranded nucleic acid molecules that have the same sequences as one another, but that have different sequences from the sequences of the single stranded nucleic acid molecules at the first location, and that are also hybridized to primers in a manner to allow primer extension in the presence of nucleotides and a nucleic acid polymerase;

c) providing each location with a nucleic acid polymerase and a given nucleotide in labelled and unlabelled form under conditions that allow extension of the primers if a complementary base or if a plurality of such bases is present at the appropriate position in the single stranded nucleic acid molecules;

d) detecting whether or not said labelled nucleotide has been used for primer extension at each location by determining whether or not the label present on said nucleotide has been incorporated into extended primers, and if said labelled nucleotide has been used in primer extension, this step involves [includes a further step of] detecting how many of said nucleotides have been used per extended primer;

e) repeating steps c) and d) one or more times so that extended primers each comprising a plurality of labels are provided;

whereby the sequence of the nucleic acid molecules at the first and second locations is obtained by reference to the number and type of nucleotides used in primer extension at these [each] location.

21. **(Twice Amended)** A method of sequencing a target nucleic acid comprising:

(a) hybridizing the target nucleic acid to a primer whereby the target nucleic acid can serve as a template for extension of the 3' end of the primer;

(b) incubating the hybridized target nucleic acid/primer with a polymerase and a type of nucleotide bearing a label under conditions supporting template-directed extension of the primer if the nucleotide type can be incorporated as the complement of a corresponding nucleotide of the target;

(c) measuring first label incorporated into the primer to determine whether, and if so, by how may base increments, the primer has been extended by incorporation of the nucleotide type;

(d) incubating the hybridized primer/target nucleic acid with a different type of nucleotide bearing a label under conditions supporting template-directed extension of the primer if the different nucleotide type can be incorporated so as to be complementary to a corresponding nucleotide in the target;

(e) measuring incremental label incorporated into the primer due to the previous incubating step to determine whether, and if so, by how may base increments, the primer has been extended by incorporation of the different nucleotide type; and

(f) repeating steps (b) - (e) so that extended primer comprising a plurality of labels are provided, until a desired portion of the target sequence can be determined from the incremental base additions to the primer.